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AWARD NUMBER: W81XWH-04-1-0749

TITLE: A Tissue Engineering Approach to Study the Progression of Breast Tumor Metastasis in Bone

PRINCIPAL INVESTIGATOR: Mingxin Che, M.D., Ph.D.
Daotai Nie, Ph.D.

CONTRACTING ORGANIZATION: Wayne State University
Detroit, Michigan 48202-3622

REPORT DATE: August 2006

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

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1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
01-08-2006	Final	30 Jul 2004 – 29 Jul 2006

4. TITLE AND SUBTITLE

A Tissue Engineering Approach to Study the Progression of Breast Tumor Metastasis in Bone

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-04-1-0749

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Mingxin Che, M.D., Ph.D. and Daotai Nie, Ph.D.

E-Mail: mche@dmc.org

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Wayne State University
Detroit, Michigan 48202-3622

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Most patients dying of breast cancer suffer painful bone metastasis. It is our hypothesis that the invasive growth and progression of breast metastatic lesions in bone requires the participation of various constituents from "soil". A reconstitution of such "soil" for the growth of breast metastatic cells will provide tremendous insights into factors critical for breast cancer growth in bone. We will firstly use our basic calcium minerals to reconstitute the mineral part of bone environment and then study the interaction of breast cancer cells with bone minerals. Then we will culture osteoblasts or bone marrow stromal cells on calcium phosphate scaffolds and then study the growth of breast cancer cells in this engineered bone microenvironment. Finally we will xenograft the calcium phosphate scaffolds, filled with cultured breast cancer cells, into athymic mice and study the resultant tumor growth and progression in vivo. The defined approach proposed will enable us to evaluate and define each individual components of bone for their role in the progression of breast bone metastasis.

15. SUBJECT TERMS
Breast cancer, bone metastasis, tissue engineering, mouse model

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			**19b. TELEPHONE NUMBER** *(include area code)*
U	U	U	UU	7	

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

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INTRODUCTION

Human breast cancers display a very high frequency of metastasis to bone. The progression of breast bone metastasis is often "osteolytic" causing a significant deterioration in the quality of life for patients. Various constituents of bone have been postulated to contribute to the progression of breast cancer metastatic lesions in bone. However, study of bone metastasis is hampered by the lack of robotic experimental models. Although animal models involving intracardiac injection of tumor cells or direct injection of tumor cells into tibia are valuable for studying breast bone metastasis, they are complicated and possess too many confounding factors for interpreting results.

We propose a defined approach to reverse engineer an environment, which breast cancer cells may encounter during their progression in bones, to study various aspects of breast bone metastasis. In this approach, we will use calcium phosphate crystals, manufactured as a 3-D scaffold, to grow breast cancer cells and study their growth and progression. We will seed and culture breast cancer cells on calcium phosphate scaffold and then study the growth and progression of breast cancer cells in this defined condition. Then we will xenograft the scaffolds, along with breast cancer cells, into mice to study the tumor formation and progression. Our approach can be extended to evaluate the postulated involvement of other constituents of bone, such as bone marrow stromal cells and osteoblasts, in the progression of breast cancer bone metastasis. For example, we can co-culture stromal cells with breast cancer cells in the scaffolds and then xenograft the scaffolds into mice to assess whether the presence of bone marrow stromal cells can promote the growth and progression of breast tumors. We believe our approach can address many unanswered questions regarding breast cancer bone metastasis.

To validate our approach, the following proof-of-concept studies are proposed:

Aim 1. Study whether calcium phosphate scaffold can directly support the growth and progression of breast cancer in vitro;

Aim 2. Study whether or not calcium phosphate scaffold can promote the formation and osteolytic progression of breast tumors in vivo;

Aim 3. Study whether osteoblasts and bone marrow stromal cells promote the growth and progression of breast cancer cells in vitro and in vivo.

BODY OF REPORT

KEY RESEARCH ACCOMPLISHMENT

Presentation at 2005 Era of Hope Meeting.

Results

Task 1. Study whether calcium phosphate scaffold can directly support the growth and progression of breast cancer in vitro.

Breast cancers display a very high frequency of metastasis to bone, causing significant deterioration in the quality of life for patients. Various constituents of bone have been postulated to contribute to bone metastasis. However, animal models involving intraosseous or intracardiac injection of tumor cells are complicated and not conducive for interpreting factors critical for bone metastasis.

In this study, we attempted to reverse engineer an environment to study various aspects of breast bone metastasis. At the initial stage of proof of concept study, we used calcium phosphate (CaPi) crystals as substrata to grow breast cancer cells. We found that MDA-MB-231 (Figure 1) and MCF-7 cells (Figure 2) can adhere, spread, and grow on CaPi substrate. The survival and growth of breast cancer cells on CaPi substrate required the activity of vacuolar ATPase, as suggested by the study using the inhibitor Bafilomycin A1 (Figure 1 and Figure 2).

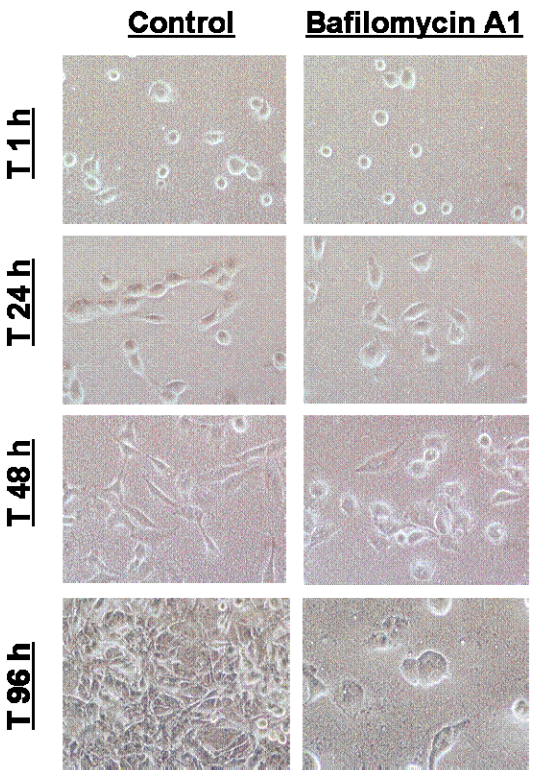

Fig. 1. Growth of MDA-MB-231 cells on CaPi (Left panel) and inhibition of cell growth by Bafilomycin A1, an inhibitor of vacuolar ATPase.

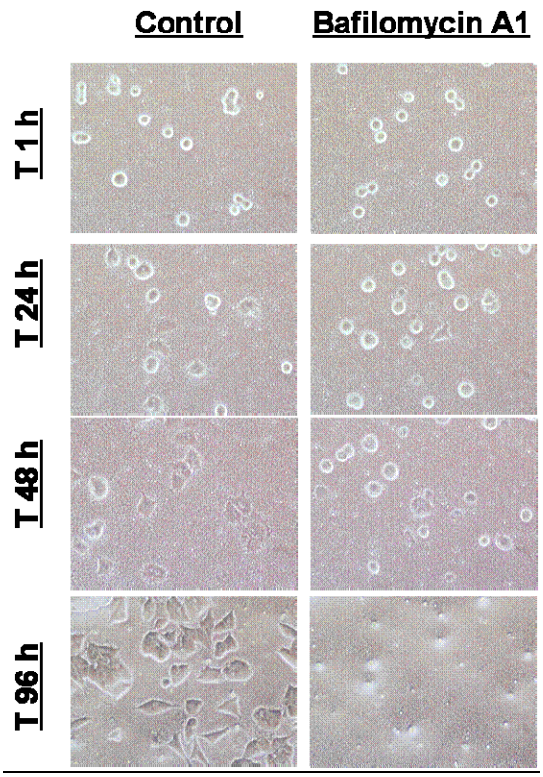

Fig.2. Growth of MCF7 cells on CaPi (Left panel) and inhibition of cell growth by Bafilomycin A1, an inhibitor of vacuolar ATPase (right panel).

Task 2. Study whether or not calcium phosphate scaffold can promote the formation and osteolytic progression of breast tumors in vivo.

We have performed xenografting the CaPi scaffolds, loaded with MDA-MB-231 cells, into mice. We observed that the presence of CaPi scaffolds irritated mice and mice had to be sacrificed much earlier than planned, making the full assessment of osteolytic progression in vivo more difficult.

As mentioned in task 1, we observed that the activities of V-ATPase, which can degrade CaPi to mobilize calcium and phosphate, were required for tumor cell growth on CaPi. Here we further assessed the expression of V-ATPase in breast carcinoma in close proximity of minerals by immunocytochemistry using bone marrow aspirates from breast cancer patients. As shown in Figure 3, V-ATPase was expressed in BCa cells in the proximity of bone matrix.

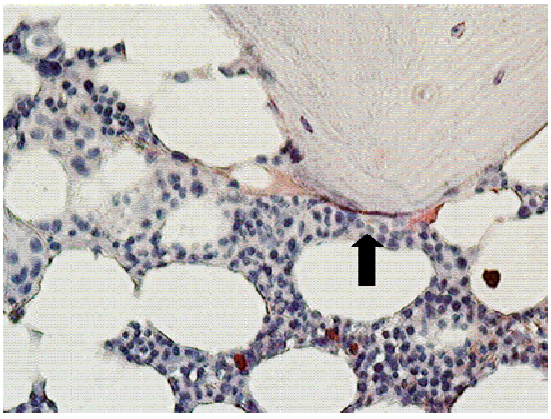

Fig.3. Expression of vacuole ATPase in breast cancer cells in the proximity of bone matrix (Positive staining was indicated by the brownish color as arrowed).

Task 3. Study whether osteoblasts and bone marrow stromal cells promote the growth and progression of breast cancer cells in vitro and in vivo.

We also cultured bone marrow mesenchymal cells, as part of our eventual goal to reconstitute an artificial bone microenviroment to study bone metastasis. Due to the irritation caused by CaPi scaffolds xenografted, mice had to be sacrificed much earlier than planned, making the full assessment of tumor progression in vivo difficult to conduct.

We also evaluated whether breast cancer cells can degrade bone (osteolytic). We measured the mobilization of calcium as an indicator for osteolytic degradation of the scaffold. As shown in Figure 4, MCF-7 cells, and to much greater extent, MDA-MB-231 cells are able to cause significant bone degradation. The results suggest that even in the absence of osteoblasts and bone marrow stromal cells, breast cancer cells can directly initiate osteolytic progression.

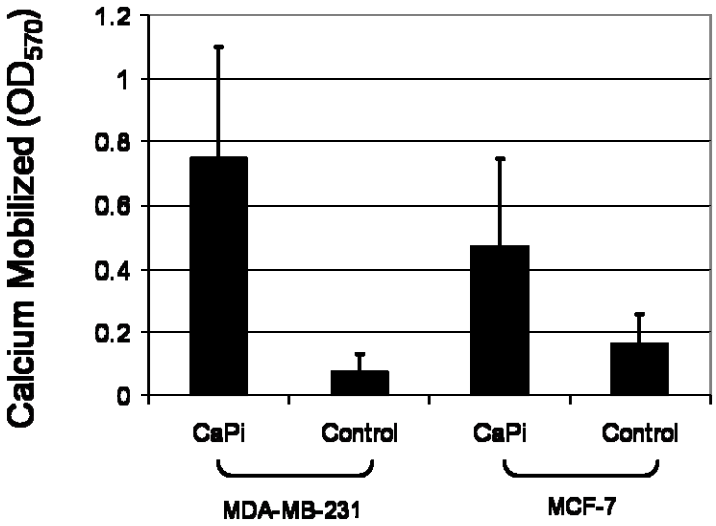

Fig. 4. Mobilization of calcium from CaPi scaffolds.

SUMMARY/CONCLUSIONS:

- CaPi supported the growth of breast cancer MDA-MB-231 and MCF7 cells.
- Vacuolar ATPase is expressed in breast carcinoma in bone marrow.
- Inhibition of ATPase by Bafilomycin A1 reduced cell survival and growth of breast cancer cells on CaPi substrate.
- Our studies using a tissue engineering approach suggest a potential role of breast cancer cells in directly causing osteolysis in bone marrow.
- Direct xonografting of CaPi scaffolds s.c. caused skin irritation. Further refinement of the scaffolds is required to use this animal model to define the factors important for breast cancer metastatic progression.

REPORTABLE OUTCOMES

- Review article published.
  Nie D, Honn KV. Eicosanoid regulation of angiogenesis in tumors. Semin Thromb Hemost. 2004 Feb;30(1):119-25.
- Abstract published.
  Krishnamoorthy, S., K. R. Maddipati, D. Nie, and K. V. Honn. 12-Lipoxygenase in hypoxia and hypoxia-induced angiogenesis. Proc. Amer. Assoc. Cancer Res. 45: #3591, 2004.
- Development of animal models: Yes.